

# **Supplementary Information**

for

## **Dimethylcysteine (DiCys)/o-phthalaldehyde derivatization for chiral metabolite analyses: cross-comparison of six chiral thiols**

Ankhubayar Lkhagva <sup>1</sup>, Hwan-Ching Tai <sup>2\*</sup>

<sup>1</sup> Department of Chemistry, National University of Mongolia, Ulaanbaatar, Mongolia

<sup>2</sup> School of Public Health, Xiamen University, Xiamen, Fujian, China

\* Corresponding Author

**Table S1.** Enantiomeric analysis of amino acids using different chiral thiols

(a)

Amino acid	OPA/DiCys	
	Resolution factor	Elution order
Ala	1.7	D, L
Glu	1.2	D, L
Phe	2.3	D, L
Ser	2.8	D, L
Tyr	1.7	D, L

(b)

Amino acid	OPA/NAC	
	Resolution factor	Elution order
Ala	1.1	D, L
Glu	0.6	D, L
Phe	0.3	D, L
Ser	1.6	D, L
Tyr	2.3	L, D

(c)

Amino acid	OPA/NAP	
	Resolution factor	Elution order
Ala	0.9	L, D
Glu	0.5	D, L
Phe	#	
Ser	1.4	D, L
Tyr	2.0	L, D

(d)

Amino acid	OPA/IBLC	
	Resolution factor	Elution order
Ala	2.9	L, D
Glu	1.6	L, D
Phe	2.2	L, D
Ser	2.7	L, D
Tyr	1.8	L, D

(e)

Amino acid	OPA/NBC	
	Resolution factor	Elution order
Ala	#	
Glu	1.6	L, D
Phe	0.7	L, D
Ser	1.4	L, D
Tyr	0.5	L, D

(f)

Amino acid	OPA/ BTCC	
	Resolution factor	Elution order
Ala	#	
Glu	#	
Phe	0.3	L, D
Ser	#	
Tyr	0.9	D, L

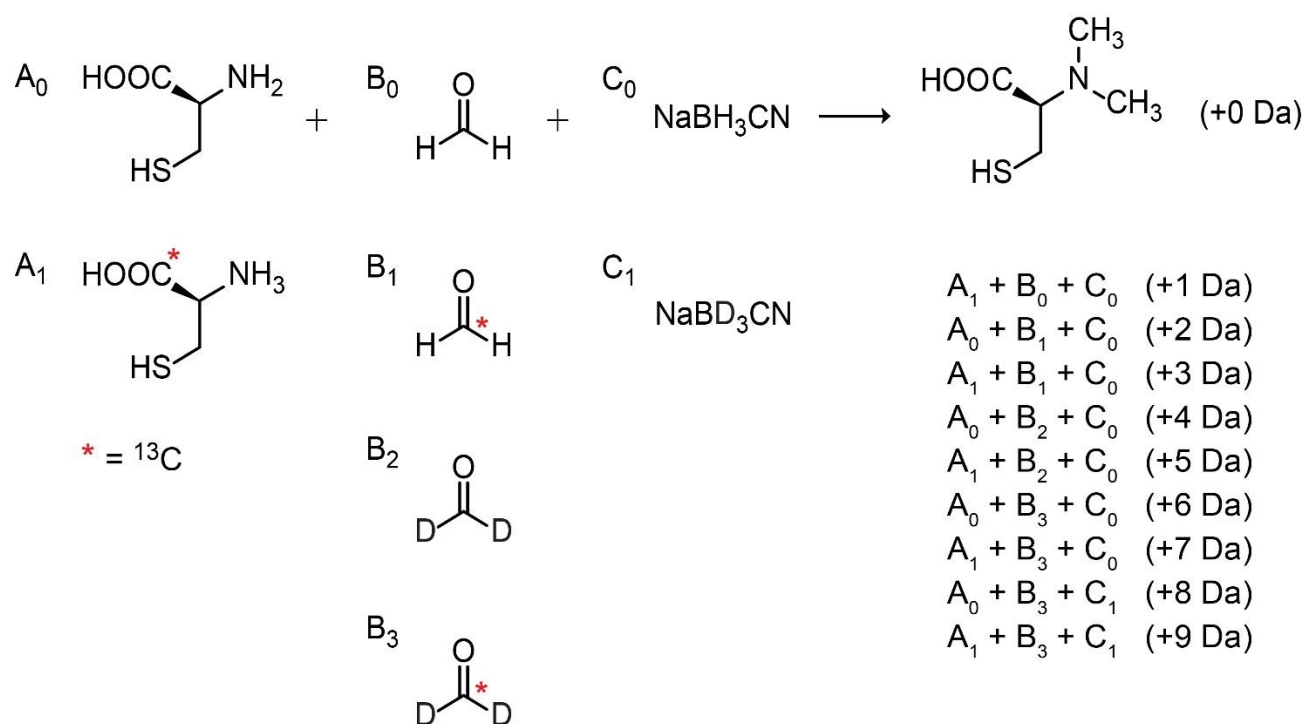
Note: Resolution factor ( $R_s$ ) =  $1.18 (t_2 - t_1)/(w_2 + w_1)$ , where  $t$  is elution time,  $w$  is the peak width at half maximum height. # indicates failure to resolve the enantiomer pair.

**Table S2.** MS/MS analysis of chiral amino acids identification in rice water by IBLC/OPA

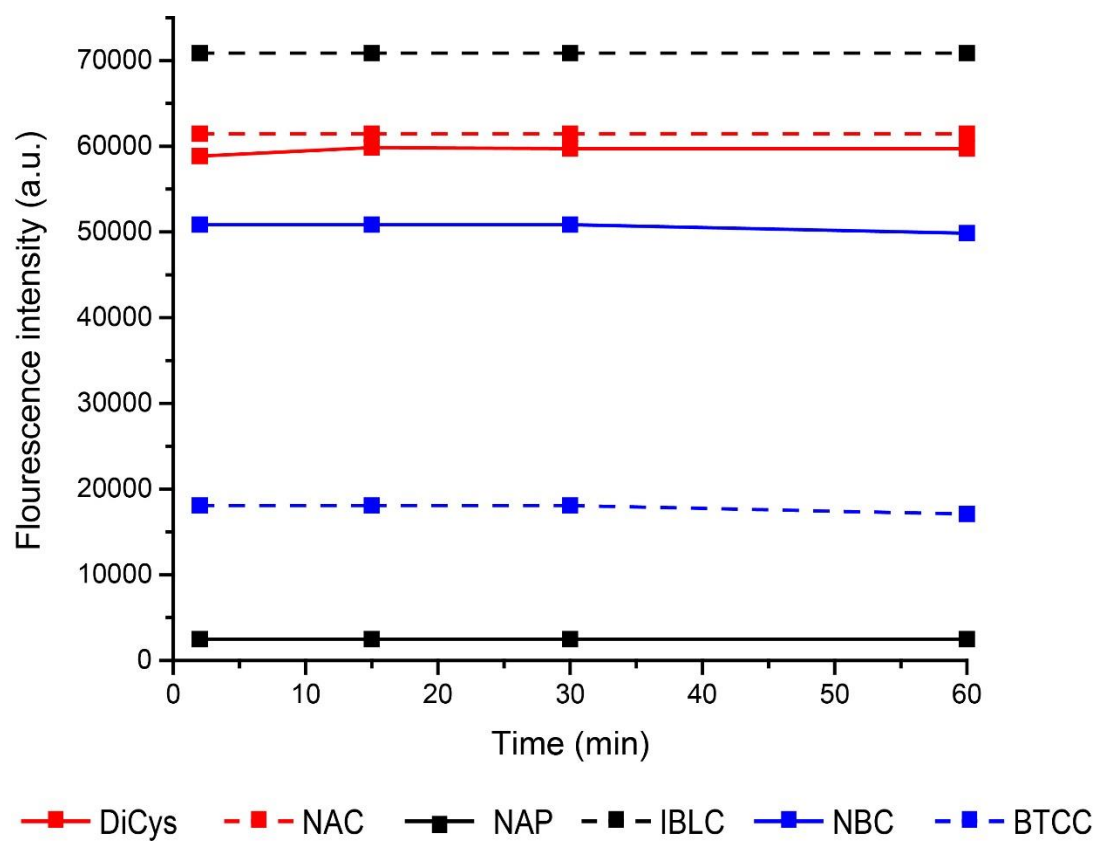
Amino acid	Retention time (min)	Precursor ion [M+H] ( <i>m/z</i> )	Manually integrated peak area (MA)	Product ion [M+H-IBLC] ( <i>m/z</i> )
L-Ala	32.96	379.31	2.62x10 <sup>7</sup>	189.07
D-Ala	34.41	379.32	2.31x10 <sup>6</sup>	189.07
L-Arg	37.14	464.73	8.98x10 <sup>6</sup>	274.13
D-Arg	38.51	464.74	1.56x10 <sup>6</sup>	274.15
L-Asn	21.71	422.23	5.06x10 <sup>6</sup>	232.09
L-Asp	6.95	423.35	1.94x10 <sup>7</sup>	233.10
L-Cys	35.26	411.23	3.01x10 <sup>5</sup>	211.03
L-Glu	8.79	437.56	2.69x10 <sup>7</sup>	247.08
L-Gln	27.62	436.34	2.31x10 <sup>6</sup>	246.10
Gly	31.12	365.01	9.67x10 <sup>6</sup>	175.06
L-His	29.47	445.09	1.95x10 <sup>6</sup>	255.16
L-Ile	43.36	421.14	9.29x10 <sup>6</sup>	231.08
L-Leu	44.87	421.43	4.32x10 <sup>6</sup>	231.08
L-Lys	42.05	436.41	1.08x10 <sup>6</sup>	246.10
D-Lys	43.93	436.40	3.31x10 <sup>5</sup>	246.10
L-Met	41.24	439.04	2.99x10 <sup>6</sup>	249.05
L-Phe	41.99	455.39	4.91x10 <sup>6</sup>	265.08
D-Phe	43.12	455.38	4.66x10 <sup>5</sup>	265.08
L-Pro	46.70	405.08	4.26x10 <sup>6</sup>	215.09
L-Ser	23.52	395.31	4.55x10 <sup>6</sup>	205.07
D-Ser	24.92	395.33	4.75x10 <sup>5</sup>	205.07
L-Thr	30.17	409.13	1.44x10 <sup>7</sup>	219.08
L-Trp	42.04	494.28	2.95x10 <sup>6</sup>	304.05
L-Tyr	36.39	471.45	3.42x10 <sup>6</sup>	281.10
D-Tyr	37.63	471.41	4.20x10 <sup>5</sup>	281.10
L-Val	40.85	407.04	7.68x10 <sup>6</sup>	217.09
D-Val	41.61	407.03	2.46x10 <sup>5</sup>	217.08

**Table S3.** MS/MS analysis of chiral amino acids identification in rice water by DiCys/OPA

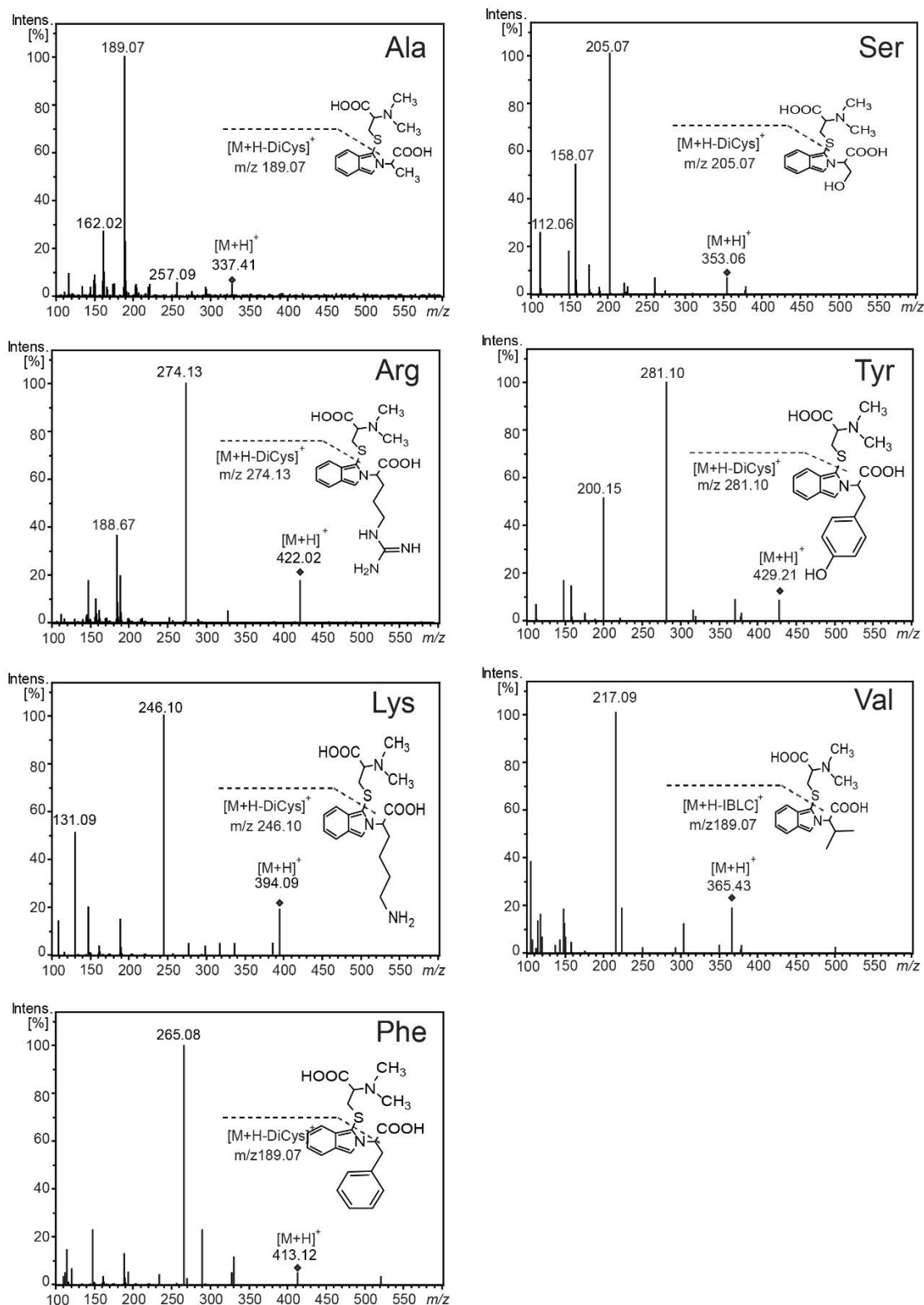
Amino acid	Retention time (min)	Precursor ion [M+H] ( <i>m/z</i> )	Manually integrated peak area (MA)	Product ion [M+H-DiCys] ( <i>m/z</i> )
L-Ala	32.51	337.41	1.75x10 <sup>7</sup>	189.07
D-Ala	29.50	337.42	1.54x10 <sup>6</sup>	189.07
L-Arg	34.27	422.02	5.99x10 <sup>6</sup>	274.13
D-Arg	33.67	422.03	1.04x10 <sup>6</sup>	274.13
L-Asn	16.05	380.21	3.37x10 <sup>6</sup>	232.09
L-Asp	6.54	381.12	1.29x10 <sup>7</sup>	233.10
L-Cys	33.09	369.23	9.50x10 <sup>5</sup>	221.28
L-Glu	7.68	395.45	1.79x10 <sup>7</sup>	247.08
L-Gln	55.81	394.32	1.54x10 <sup>6</sup>	246.10
Gly	22.51	323.08	6.44x10 <sup>6</sup>	175.06
L-His	24.88	403.43	1.30x10 <sup>6</sup>	255.16
L-Ile	40.88	379.21	6.19x10 <sup>6</sup>	231.08
L-Leu	41.07	379.26	2.88x10 <sup>6</sup>	231.08
L-Lys	45.81	394.09	7.21x10 <sup>5</sup>	246.10
D-Lys	44.17	394.08	2.21x10 <sup>5</sup>	246.10
L-Met	35.68	397.32	1.99x10 <sup>6</sup>	249.05
L-Phe	43.22	413.12	3.27x10 <sup>6</sup>	265.08
D-Phe	42.14	413.13	3.11x10 <sup>5</sup>	265.08
L-Pro	24.39	363.33	2.84x10 <sup>6</sup>	215.09
L-Ser	17.43	353.06	3.03x10 <sup>6</sup>	205.07
D-Ser	16.31	353.07	3.17x10 <sup>5</sup>	205.07
L-Thr	29.22	367.13	9.60x10 <sup>6</sup>	219.08
L-Trp	38.82	452.24	1.96x10 <sup>6</sup>	304.05
L-Tyr	37.13	429.21	2.28x10 <sup>6</sup>	281.10
D-Tyr	35.93	429.23	2.80x10 <sup>5</sup>	281.10
L-Val	36.52	365.43	5.12x10 <sup>6</sup>	217.09
D-Val	35.29	365.42	1.64x10 <sup>5</sup>	217.08



**Figure S1.** Proposed synthesis scheme for isotope-tagged DiCys.

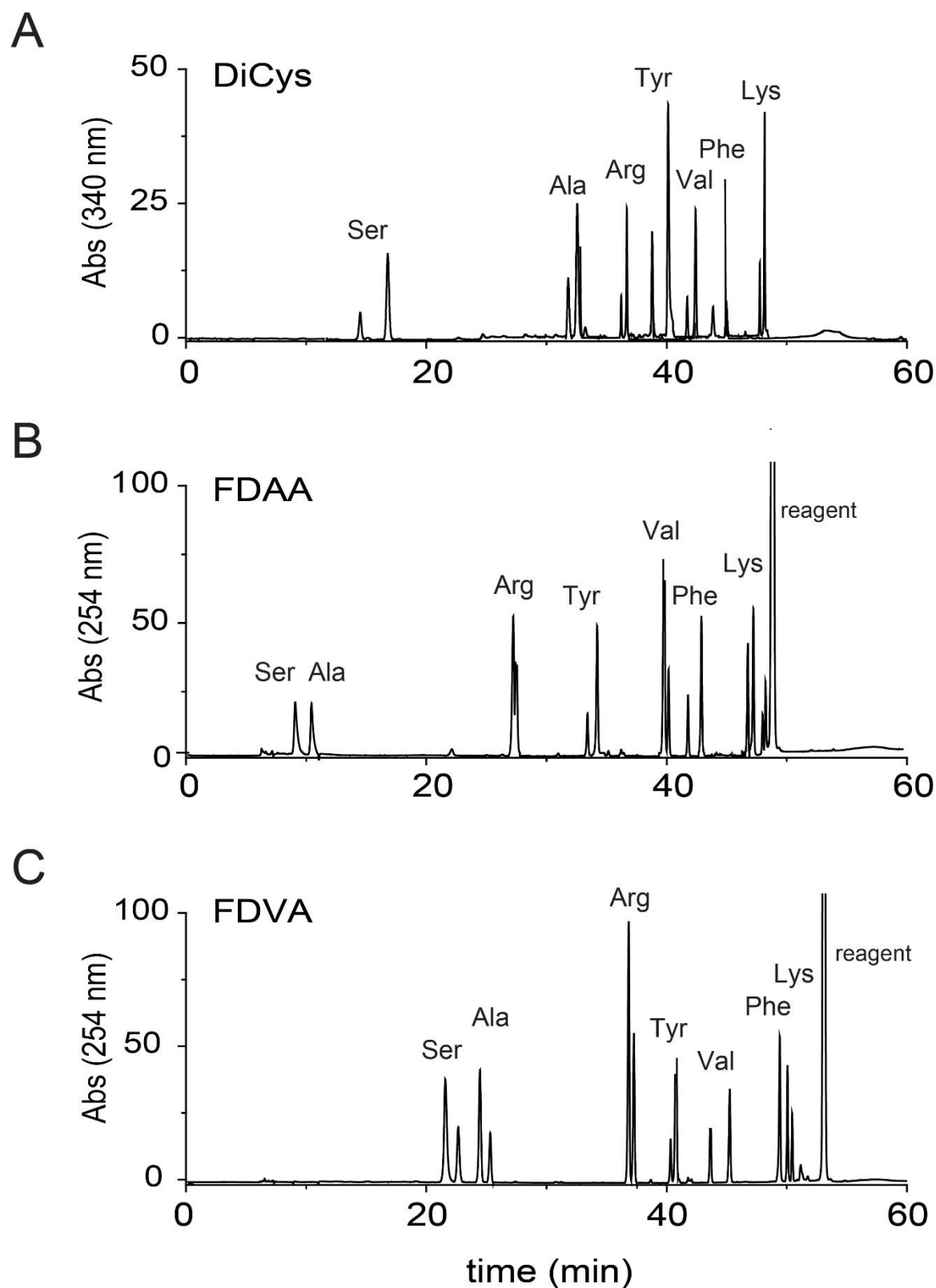


**Figure S2.** The stability of OPA-derivatized alanine with different thiols at 4 °C.



**Figure S3.** Fragmentation patterns of DiCys/OPA adducts with seven D-amino acids





**Figure S4.** RP-HPLC analysis of amino acid enantiomers derivatized with OPA/DiCys (A) and Marfey's reagents—FDAA (B), and FDVA (C). The ratio between L: D amino acids is 3:1.